On page 61, line 7, change "radionucloids" to --radionuclides--.

In the Claims:

Claim 13, line 1, after "the" and before "cells", insert --tumor--.

Claim 18, line 1, after "polypeptide", insert --subunit--.

In the Figures:

Drafting

In Figure 7, please insert immediately outside the upper right-hand corner of the upper graphic profile --A--, insert immediately outside the upper right-hand corner of the lower graphic profile --B--. Applicants have attached hereto as Exhibit 0 a copy of Figure 7, corrected in red ink and a copy of new Figure 7.

REMARKS

The amendments to the specification and to Figure 7 do not constitute the addition of new matter. Support for said amendments may be found as follows.

The amendment to the brief description of Figure 7, on page 10, and the corresponding amendment to Figure 7 merely clarify the original description as requested by the Examiner in the Office Action issued May 18, 1992, and does not constitute new matter. Applicants further direct the Examiner to Example XV on pages 36 and 37 of the specification which describes the experiment yielding the data shown in Figure 7. The addition of "A" and "B" to Figure 7, merely corrects an obvious oversight

and, additionally, clarifies the "before" and "after" depictions set forth in the Brief Description and also in Example XV. The amendments to pages 4, 5, 6, 24, 25, 36, 40, 54, 60 and 61 simply correct typographical and grammatical errors.

Claims 1-46 are pending in the subject application. Claims 2-5, 7-10 and 20-46 are withdrawn from consideration as being drawn to a non-elected invention. Claims 13 and 18 have been amended to further clarify the scope of the invention and do not raise an issue of new matter. Accordingly, claims 1, 6 and 11-19 are currently under examination. Claims 1, 6 and 11-19 stand variously rejected under 35 U.S.C. §§ 102, 103 and 112. The specification stands objected to under 35 U.S.C. § 112, first paragraph, as failing to provide an enabling disclosure. Applicants have reviewed the grounds for the rejections, but traverse for the reasons set forth below.

I. Rejections Under 35 U.S.C. § 112

The specification stands objected to under 35 U.S.C. § 112, first paragraph, as allegedly failing to provide an enabling disclosure. According to the Examiner, the specification fails to teach substantially pure subunits of UTAA. Claims 1, 6 and 11-19 are similarly rejected upon these grounds.

Applicants submit the specification clearly defines "substantially pure subunits of UTAA." On page 15 of the specification, Applicants provide specific parameters of the metes and bounds of "substantially purified subunit." Specifically, a "substantially purified" subunit of U-TAA is one that, after **r duction** by ß-mercaptoethanol and **separation** by SDS-PAGE, has a molecular weight of about 90-100 kD; after **purification** by DEAE Sephacel anion exchange chromatography is

heat stable, has a molecular mass in the range of 590-620 kD under non-reducing conditions and an isoelectric point of 6.1. Specific examples of the purification schemes are presented in Examples I and II on pages 22-24 which set forth gel filtration which retained a molecule that reacts with antibody, while removing nonreactive molecules. Applicants contend that, given the above-mentioned parameters and examples, a person of skill in the art would possess sufficient ability to make and use the subject invention.

In response to the Examiner's query comparing the results of silver staining and immunoblotting, Applicants maintain that the specific differences observed by the Examiner are not dispositive of the degree of purity of the U-TAA analyzed thereon. Specifically, a person of skill in the art would acknowledge that the motivations behind analysis via immunoblotting versus analysis via silver staining are distinct. Immunoblotting is specific for a particular antigen of interest, whereas silver staining analysis functions as a general protein detection technique. It is well known in the art that silver staining methods result in high backgrounds due to non-specific dye adsorption (see Harlow E. et al., Antibodies A Laboratory Manual; Cold Spring Harbor Laboratory, pp 93. (1988). The background problem is inherent in this particular staining technique. The silver stain is known to frequently produce artifacts on the gel which are the result of non-protein chemical contaminants that react with the silver reagent used in the stain. These contaminants are frequently located in sample buffers and running buffers and are difficult to eliminate. Therefore, a comparison of the results of two distinct techniques has no logical relevance on the degree of purity of a given sample. In support of this assertion, Applicants direct Examiner to Example XIV on page 36, wherein Applicants subsequently

transferred the SDS-PAGE gel which had been silver stained onto nitrocellulose paper and immunostained. This subsequent immunostaining revealed that the 90 kD band was the **only one** which reacted with both murine monoclonal antibody, AD1-4OF4, and baboon polyclonal anti-U-TAA IgG.

The Examiner alleges that the specification fails to teach reagents that are reactive with antibodies reactive to U-TAA. Applicants respectfully traverse this objection and direct the Examiner to page 17, lines 15-22 defining said reagents. However, the Examiner also acknowledges that Applicants provide in Example XXXIV, on pages 60 and 61 of the specification, a protocol for the preparation of human anti-idiotypic antibodies. Basically, antibodies react with antigens. An antigen molecule consists of determinants or epitopes which are specific to the idiotopes of an antibody molecule. It is well known that it is possible to produce anti-idiotype serum which reacts with one or more idiotopes on the immunizing antibody. Further it is also clear that an anti-idiotype can function as a substitute for the original antigen, i.e., either stimulating or depressing the immune response (see Jerne, N.K., "Towards a network theory of the immune system", Annals of Immunology, Paris p. 373 (1974); Roitt I.M. et al., Immunology, J.B. Lippincott Co., Philadelphia; p. 10.1-10.6 (1989); Eichman et al., CRC Critical Reviews in Immunology, 7:193-227 (1987)). Contrary, to the Examiner's contention, Applicants maintain that, given the state of the art and the guidance provided in Example XXXIV of the subject application, a person of skill in the art would possess the information necessary to make and use the subject invention. The courts have made it clear that there is no obligation to provide a large number of examples in order to establish satisfaction of the enablement requirements and, therefore a specification with no actual examples may comply. See In re Robins, 429 F.2d 452,

456-57, 166 USPQ 552, 555 (CCPA 1970)("[R]epresentative examples are not required by the statute and are not an end in themselves.") Applicants strongly disagree with the Examiner's allegation that "production of any specific antibody is speculative, at best, and in the case of anti-idiotype antibodies even more so." Applicants direct the Examiner to Example XXXII, reporting the in vitro production of human antibodies to UTAA, in particular LCL subclones 2.6 and 2.11 which produce anti-UTAA and anti-FA IgM antibodies. These subclones, after subjection to several rounds of further subcloning will ultimately result in monoclonal species.

The Examiner states that the specification fails to teach vaccine formulations containing cells with U-TAA on their surface in addition to either GM-2, GD-2, fetal antigen or M-TAA in a pharmaceutically acceptable carrier. Additionally, the Examiner contends that it is unclear from the specification exactly what GM-2, GD-2, fetal antigen and M-TAA are.

In response to the Examiner's contention, Applicants direct the Examiner to page 19, line 15 through page 20, line 31, wherein the melanoma tumor vaccine is characterized. Further, beginning on page 41, Example XX, Applicants describe the test and control subjects that were immunized with a melanoma tumor vaccine. Applicants specifically detail the vaccination protocol and subsequent titer analysis on page 42, lines 10-37, and pages 43 and 44 followed by a graphic representation of the aforementioned analysis. Applicants, therefore, clearly teach vaccine formulations containing cells with U-TAA on their surface in addition to either GM-2, GD-2, fetal antigen or M-TAA, e.g., melanoma cell lines UCLA-SO-M10, UCLA-SO-M24 and UCLA-SO-M101. The aforementioned tumor cells were used in said vaccine which was administered to patients who were monitored for and exhibited

increased levels of antibody to UTAA.

To address the Examiner's unclarity as to the meaning of GM-2, GD-2, fetal antigen and M-TAA, Applicants direct the Examiner to page 16, lines 30-32, defining the aforementioned group as "tumor associated antigens." A person of skill in the art to which the subject invention pertains would be familiar with these well-defined tumor antigens. In fact, these tumor associated antigens are noted and supported by various references cited in a reference cited by the Examiner against the subject application, e.g., Euhus, et al., "Induction of Antibodies to a Tumor-Associated Antigen by Immunization with a Whole Melanoma Cell Vaccine", Canc. Immunol. Immunother., 29:247-254 (1989).

In response to the Examiner's allegations that the specification fails to teach that the administration of any vaccine that inhibits cancer in the recipient, Applicants direct the Examiner to the study conducted wherein melanoma patients were vaccinated with inactivated tumor cells having UTAA on the cell surface and at least one tumor associated antigen (see Examples XX-XXIV). Applicants have demonstrated that such vaccination induces anti-UTAA antibodies of both the IgM and IgG isotypes. Applicants further report two- to five-fold increases in anti-U-TAA IgM titers in 11 of the 15 patients and in anti-U-TAA IgG in 6 of the 15 patients. Applicants again direct the Examiner to the Euhus reference "Induction of antibodies to a tumor-associated antigen by immunization with a whole melanoma cell vaccine" in Cancer Immunol. Immunother. 29: 247- 254 (1989).

> "This MCV is composed of three allogeneic cell lines chosen for their expression of four well-defined tumor antigens. These include two ganglioside antigens, GM-2 and GD-2, a glycoprotein, termed fetal antigen. and a lipoprotein antigen known as melanoma-tumor-associated antigen (M-TAA). . . . there is evidence that other tumor antigens are also expressed. One such antigen is

> a melanoma urinary-tumor-associated antigen (U-TAA)." (p. 247) "[M]elanoma U-TAA is present on the surface of cultured melanoma cells and administration of a whole irradiated melanoma cell vaccine induces anti-(U-TAA) antibodies of both the IgM and IgG isotypes." (p. 253)

Additionally, Applicants have shown that this antibody response was not diminished in advanced stages of the disease (see Example XXVII). Applicants have clearly demonstrated that vaccination with MCV markedly increases the patients antibody production against UTAA. With this in mind, Applicants direct the Examiner to the definition of "inhibit" on page 17, lines 5-13 of the specification.

> "The antibody produced in the individual after administration of the vaccine inhibits or treats the cancer, for example a melanoma. Inhibiting the cancer refers to the ability to contact the tumor cells with a reagent which can prevent the cells from proliferating, thus resulting in cell death and a reduction in size of the tumor. Alternatively, inhibiting can include a direct cytotoxic effect on the tumor cells."

Once the antibody-antigen complex is formed, the patient's immune response begins the process of eliminating antigen. Therefore, Applicants do teach the administration of a "vaccine" that "inhibits" cancer in the recipient.

The Examiner alleges that the specification fails to teach a vaccine containing the composition of claim 1. The Examiner further states that "[n]owhere does the specification teach immunization with substantially purified material. Additionally, the specification does not teach that the administration of pure protein results in antibody production against tumor cells."

Applicants respectfully traverse and direct the Examiner to the discussion of the substantially purified polypeptide subunit of UTAA in the second paragraph of § I of this Communication. Applicants reaffirm their position stated therein. Contrary to

the Examiner's assertion, Applicants do teach 'immunization with substantially purified subunit.' Specifically, in Example III, Applicants set forth the experimental details of the production of xenogeneic anti-U-TAA serum in a baboon. The baboon was injected intramuscularly with the "substantially purified subunit" produced in Example I. The antigen prepared in Example I "was used as . . . immunogen for production of xenoantibody and murine monoclonal antibody." (see pg. 23, lines 7-10). The antibodies produced by the baboon in response to said immunogen, were found to have a cytotoxic effect on various human cultured tumor cell lines (see pgs. 26-27). Applicants further detail in Example V, the immunization of a mouse with the "substantially purified material" produced in Example I, which resulted in the isolation of a murine monoclonal antibody, AD1-40F4, capable of differentiating between U-TAA and normal human sera. Applicants attach hereto as Exhibit 1, copies of pages 235-236 of Stenesh, J. Dictionary of Biochemistry and Molecular Biology, 2d Ed., John Wiley & Sons, New York (1989). Specifically, Applicants direct the Examiner to the definitions of "immunization" and "immunogen." To wit:

> "immunization 1. The administration of an antigen to an animal organism to stimulate the productions of antibodies by that organism."
>
> "immunogen 1. ANTIGEN. 2. A substance capable of producing an immmune response that leads to the synthesis of antibodies."

Clearly, Applicants teach immunization with the substantially purified material described in Example I which results in antibody production against tumor cells.

The Examiner alleges that the specification fails to provide an adequate written description of the invention, fails to provide an enabling disclosure and fails to present the best mode on the ground that the biological materials have not been

deposited.

Applicants respectfully traverse on the grounds set forth by the CAFC in Amgen, Inc. v. Chugai Pharmaceuticals Co. Ltd, 18 USPQ2d 1016, 1025 (Fed. Cir. 1991). The Court in the Amgen case held "if the cells can be prepared without undue experimentation from known materials, based on the description in the patent specification, a deposit is not required." Applicants maintain that a person of skill in the art would possess the ability to produce the cell lines from known materials using the description set forth in the specification.

Claims 1, 6, 13 and 18 stand rejected under 35 U.S.C. §112, second paragraph as being indefinite for failing to particularly point our and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner finds the terminology "substantially purified" in claim 1, "reagents" and "reactive" in claim 6 unclear. Further, the Examiner inquires as to the antecedent basis of "the cells" in claim 13 and "the polypeptide" of claim 18.

In response to the Examiner's unclarity as to the description of "substantially purified", Applicants direct the Examiner to the discussion set forth in §I, second paragraph of this Communication setting forth citations from the specification of the subject application describing the parameters of "substantially purified." Applicant maintain that a person of skill in the art, given the parameters set forth in the subject application would not be unclear as to the aforementioned terminology. Applicants clearly set forth the definition of "reagents which are reactive. . . ." Applicants direct the Examiner to the discussion set forth in §I, fourth paragraph of this Communication, specifically pointing out and defining

reagents which are reactive, and thus maintain that a person of skill in the art upon reading the subject application would know the meaning of reagents which are reactive.

Without conceding the correctness of the Examiner's position, Applicants have amended claims 13 and 18 to clarify the scope of the claims in the interest of advancing prosecution of the subject invention.

In view of the amendments and remarks set forth above Applicants respectfully request that the Examiner reconsider and withdraw the objections to the specification and rejection of the claims under 35 U.S.C. §112.

II. Rejections Under 35 U.S.C. § 102

Claims 1, 6, 11-19 stand rejected under 35 U.S.C. §102(a) as being clearly anticipated by Euhus et al. (1989). Applicants attach hereto as Exhibit 2, a copy of the corrected filing receipt which properly identifies all inventors of the subject invention. Exhibit 2, thus moots the rejection under 35 U.S.C. §102(a).

Claims 1 and 6 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Huth et al. The Examiner states that Huth et al. teach the purification of antigens found in the urine of sarcoma patients, that is subsequently concentrated and affinity purified.

Applicants traverse the Examiner's allegation. Huth et al. teach the affinity purification of an antigen having an SDS-PAGE determined molecular weight of about 40-50 kD. Applicants teach a substantially purified antigenic polypeptide subunit of UTAA

having a molecular weight of about 90-100 kD and reagents which are reactive with antibodies reactive therewith. Further, Huth et al. present no evidence whether the 40-50 kD antigen was reactive with patient antibodies. Applicants submit Huth et al. does not anticipate the subject invention.

Claims 1 and 6 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Rote et al. (1980). Specifically, the Examiner states that Rote et al. describe purification schemes of tumor associated antigens found in the urine and further centrifugation, concentration and gel filtration.

Applicants traverse the Examiner's rejection. Rote et al. teach a general purification of tumor associated antigens. Applicants teach a substantially purified antigenic polypeptide subunit of UTAA having a molecular weight of about 90-100 kD and reagents which react with antibodies reactive therewith. Rote et al. do not define any antigen allegedly purified, nor was a molecular species identified. Further, Rote et al. failed to make antibodies reactive to any antigen. Rote et al. merely report the results of a crude assay employing complement fixation. The experiments described by Rote et al. are known to present factors that often result in false positives. For example, Rote et al. used Sepharose-6B to purify samples. In contrast, Applicants employed Sephacryl S-200 which results in a cleaner separation and more purity of the sample. Rote et al. simply focused on the incidence of tumor associated antigens. Applicants contend that the subject invention is not anticipated by Rote et al.

Claims 1 and 6 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Real et al. The Examiner states that Real et al. teach the purification of a 90 kD glycoprotein tumor

antigen and the autologous antibodies which recognize the FD determinant on this antigen.

Applicants contend that the subject invention is not anticipated by Real et al. The subject invention is an antigenic polypeptide subunit of UTAA having a molecular weight of about 90-100 kD purified from the urine of melanoma patients and reagents which are reactive with antibodies reactive with UTAA. In contrast, Real et al. reports autologous antibodies which recognize the FD determinant on the antigen. The antigenic determinants detected by the subject invention are selected from the group of GM-2, GD-2, fetal antigen or melanoma tumor associated antigen. Real et al. does not test urine of melanoma patients, nor produce murine monoclonal antibodies to the antigen. The subject invention is immunogenic in both mouse and human. Further, the Real et al. antigen has an isoelectric point of 5.5. In contrast, the subject invention has an isoelectric point of 6.1. Applicants maintain that Real et al. does not and cannot anticipate the subject invention.

Claims 1, 6, 18 and 19 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Brown et al. The Examiner states that Brown et al teach the sequence and purification of a melanoma antigen p97. The Examiner states that it is unclear if the p97 of Brown et al is the same as the 90-100 kD protein of the instant application.

Applicants traverse the Examiner's rejection for the reasons which follow. p97 is a sialoglycoprotein which is structurally related to serum transferrin. The gene for p97 has been cloned and sequenced (see Rose, T.M. et al., Proc. Natl. Acad. Sci. USA, 83:1261-1265 (1986)). Applicants maintain that the subject invention is novel over Brown et al.

Claims 11 and 14-17 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by the 1987 Gupta et al. abstract. The Examiner states that Gupta et al. teach the vaccination of melanoma patients with tumor cells, at least one of which expresses melanoma tumor associated antigen. The Examiner is of the opinion that the cell line also produces UTAA in light of the disclosure in the 1984 Gupta et al abstract.

Applicants respectfully traverse the Examiner's rejection for the reasons which follow. For a reference to anticipate under § 102, every element of the claimed invention must be identically disclosed. Applicants submit that the 1987 Gupta et al. abstract does not anticipate the subject invention under 35 U.S.C. § 102(b). The subject invention is an antigenic polypeptide subunit of UTAA having a molecular weight of about 90-100 kD purified from the urine of melanoma patients and reagents which are reactive with antibodies reactive with UTAA. The Gupta abstract teaches a lipoprotein of 180 kD, identified as M-TAA. Thus, Gupta et al. (1987) does not anticipate the subject invention.

In light of the remarks set forth above, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of the claims under 35 U.S.C. § 102.

III. Rejections under 35 U.S.C. § 103

Claims 1 and 6 stand rejected under 35 U.S.C. § 103 as allegedly unpatentable over the 1988 Euhus et al. abstract. The Examiner is of the opinion that it would have been obvious to affinity purify the UTAA using the monoclonal antibody AD1-40F4 disclosed in said abstract. The Examiner further alleges that the motivation for doing so would be the use of the purified

material to produce polyclonal antisera to the protein for use in diagnostic procedures.

Applicants respectfully traverse the Examiner' rejection. Specifically, there is no teaching or suggestion in the Euhus et al. abstract to affinity purify UTAA. A person of skill in the art is familiar with the delicate nature of the UTAA IgM antibody. The IgM antibody is known to be particularly difficult to affinity purify resulting from its rapid inactivation once loaded onto a column. The technology available at the time the Euhus abstract was published did not permit successful purification of the IgM antibody. Further, the Euhus abstract does not teach or suggest the subject invention. The subject invention is an antigenic polypeptide subunit of UTAA having a molecular weight of about 90-100 kD purified from the urine of melanoma patients and reagents which are reactive with antibodies reactive with UTAA. The Euhus abstract merely teaches a murine monoclonal antibody to UTAA and does not suggest any further purification steps.

Claim 12 stands rejected under 35 U.S.C. § 103 as unpatentable over Wong et al. and the 1987 Gupta et al. abstract. The Examiner is of the opinion that it would have been obvious to use any known melanoma cell line, including M10, which expresses the appropriate antigens, to do so would confer the appropriate antibody response.

Applicants traverse the Examiner's rejection for the reasons which follow. The subject invention is a vaccine comprising tumor cells having UTAA on the cell surface and at least one tumor associated antigen selected from the group consisting of GM-2, GD-2, fetal antigen, or melanoma tumor associated antigen, the tumor cells being selected from the group consisting of M10,

M24, and M101. Applicants submit that the Wong reference merely affirms the presence of M-TAA on melanoma cell lines in culture, Wong does not teach or suggest the presence of U-TAA. Wong et al., in fact, teach that M24 does not express M-TAA. Further, there is no teaching or suggestion in the Gupta abstract indicating that the melanoma cell line M24 would be a suitable candidate for vaccine purposes. Applicants maintain that the subject invention is not rendered obvious by the cited references alone or in combination.

Claim 13 stands rejected under 35 U.S.C. § 103 as allegedly unpatentable over Wong et al. and Gupta et al. (1987) as applied to claim 12, and further in view of Bystryn et al. The Examiner states that Bystryn et al. disclose that transplantation antigens are undesirable contaminants in melanoma vaccines derived from tumor cells. The Examiner alleges that it would have been obvious to use tumor cells which expressed HLA antigens identical to those found in the recipient in order to avoid an immune response from the recipient to such antigens.

Applicants respectfully traverse the Examiner's rejection. Applicants reassert the discussion set forth above concerning the lack of teaching and or suggestion in the Wong et al. and Gupta et al. (1987) references to render the subject invention obvious. Applicants maintain that there is no incentive, teaching or suggestion of HLA matching in Bystryn et al. Applicants further assert that the state of the art of HLA matching at the time of the publication of the Bystryn et al. reference was not advanced to the point where HLA matching was a feasible alternative. Applicants submit that the subject invention is patentable over Bystryn et al.

In view of the remarks set forth above, Applicants respectfully request that the Examiner reconsider and withdraw the rejections of the claims under 35 U.S.C. § 103.

Summary

In summary, Applicants believe that the claims as amended, in light of the remarks herein, are in condition for allowance and respectfully request a notice to this effect. If any questions or issues remain, the Examiner is invited to contact Cathryn Campbell, at (619) 535-9001, or facsimile (619) 535-8949.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231 on 10·19·92
Date

By Antoinette F. Konski
Antoinette F. Konski, Reg. 34,202

October 19, 1992
Date of Signature